

Paragraph 53

A2
A 158 bp DNA fragment of HLA-A locus was amplified using sense primer 5' A200A and antisense primer 3' A322-1 with various genomic DNA samples obtained from UCLA registries (UCLA 210, UCLA 230 and UCLA 243). The 158 bp fragment was produced for this example using standard amplification methods. Primers used to amplify both Homo and Heterozygous DNAs in this example were (SEQ ID NOS: 1-2):

5' A200A 5' -ACA GCG ACG CCG CGA GCC A- 3' position 182 - 200, sense primer
3' A322-1 5' -CCTCGCTCTGGTTGTAGTA- 3' position 322 - 340, antisense primer

Paragraph 57

A3
Using the single base extension reaction in an attempt to capture a specific allele; Allele Specific PCR was performed using Primer Mixes (PM), H001 and H002. These two primer mixes were used for the incorporation of specific bases at the site of the polymorphism. Both PM used a common 5' primer (agcgagcgccgagcca, SEQ ID NO: 3), but used an allele specific 3' primer. PM H001 specifically incorporated the "C" (ccaagagcgcgaggtcctcg, SEQ ID NO: 4) base whereas PM H002 was specific for "A" (ccaagagcgcgaggtcctct, SEQ ID NO: 5) at the respective sites of polymorphism, when a heterozygous DNA was used.

Paragraph 68

A4
Different oligonucleotides for specific polymorphisms of the HLA A Locus were coupled to different bead sets (Luminex) to be used in the hybridization assay. The template that hybridized to the oligo coupled beads was selected to provide perfect sequence homology. Coupling beads to specific oligos was performed according to the manufacturer's instructions (Luminex Corp.). The Luminex bead-probe conjugate were hybridized with PCR fragments produced above. The sequence of the probes used for separation of allele specific PCR fragments was (SEQ ID NOS: 6-7):

L5'A107A 1AGGTATTTCTACACCTCCGTG
L5'A107C 1AGGTATTTCTCCACATCCGTG